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High-throughput *in vitro* ischemia-reperfusion model with real-time monitoring of cellular oxygenation and reactive oxygen species generation

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Ischaemia-reperfusion (IR) injury is a feature of multiple diseases including myocardial infarction, renal failure and stroke, occurring when tissue blood supply is restricted and subsequently restored. While reperfusion is essential for tissue survival, it is also associated with significant ROS-mediated damage, triggering inflammatory responses and ultimately cell death. The rational development of targeted therapeutic interventions require both a detailed understanding of the mechanisms underlying reperfusion injury and a convenient means of assessing the efficacy of putative therapeutics. This need has driven the development of a number of *in vitro* IR models including pharmacological ATP depletion, oil overlays to induce auto-hypoxia, glucose oxidase addition to cause sample deoxygenation, and N_2 purged hypoxia chambers to induce a slow reduction in dissolved oxygen.

The utility of these models has however been limited as they do not facilitate the induction of the rapid, controlled, transient, ischemic shock and reperfusion necessary to replicate IR injury condition *in vitro*. Critically, neither do they facilitate real-time cellular oxygenation monitoring to allow accurate IR characterisation or parallel measurements of critical IR injury parameters such as ROS and mitochondrial membrane potential (MMP). Here we present a model which addresses these limitations through the combined use of iPS-derived cardiomyocytes (Cor.4U®), plate reader with integrated atmospheric control facilitating rapid [O_2] modulation (CLARIOstar®) and a novel intracellular probe capable of reporting cellular oxygenation in real time (MitoXpress® Intra). Oxygenation monitoring facilitates precise control of an ischemic insult whereby instrument [O_2] can be modulated (20% to 0.1% O_2) to provide the desired depth and duration of hypoxia. The chamber can then be vented to model rapid reperfusion (1 to 10% in <10min), with parallel monitoring of ROS and MMP, facilitating detailed metabolic characterisation of the short-term metabolic implications of reperfusion and the efficacy of model therapeutic intervention.